P-CHLOROPHENOL REMOVAL IN SEQUENCING BATCH BIOFILM REACTOR

MUNA MASTURA BINTI MOHAMAD

UNIVERSITI SAINS MALAYSIA

2011

p-CHLOROPHENOL REMOVAL IN SEQUENCING BATCH BIOFILM REACTOR

by

MUNA MASTURA BINTI MOHAMAD

Thesis submitted in fulfillment of the requirements for the degree of Master of Sciences

DECEMBER 2011

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my main supervisor, Dr. Amat Ngilmi Ahmad Sujari, my co-supervisor Professor Lim Poh Eng and Associate Professor Dr. Seng Chye Eng for their guidance and continuous support during the completion of my Master study.

I am also heartily thankful to Mr. Yee, Mr. Siva, Mr. Sobri and Mrs. Norhayati for their guidance and help during my research time. I would like to express my humble gratitude to my friends, Vanitha, Jun Wei, Siok Moi, Si Ling, Siew Ling, Kwok Yii, Waheeba and members in the lab for their help and support.

I would like to express my special thanks to my family especially my parents for all their love, encouragement and supporting me spiritually throughout my life. Lastly, I would like to thank to all those who supported me in any respect during the completion of my project.

TABLE OF CONTENTS

CHAPTER 2: MATERIALS AND METHODS

CHAPTER 3: RESULTS AND DISCUSSION

APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATION

AN	Ammoniacal Nitrogen
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FT-IR	Fourier Transform Infrared Spectrometer
HPLC	High Pressure Liquid Chromatography
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
NO_2^- -N	Nitrite nitrogen
NO_3^- -N	Nitrate nitrogen
NO_x^- -N	Oxidized nitrogen
PCC	*p*-Chlorocatechol
PCP	*p*-Chlorophenol
PNP	*p*-Nitrophenol
PTFE	Polytetrafluoroethylene
RB1	SBBR with 3% (v/v) of Polyurethane Foam Cubes
RB2	SBBR with 5% (v/v) of Polyurethane Foam Cubes
RC	SBR as Control Reactor
SBR	Sequencing Batch Reactor
SBBR	Sequencing Batch Biofilm Reactor
SEM	Scanning Electron Microscope
SND	Simultaneous Nitrification and Denitrification
SS	Suspended Sludge
SV_{30}	Settled Sludge Volume
SVI	Sludge Volume Index

TOC	Total Organic Compound
UASB	Upflow Anaerobic Sludge Blanket
UV-VIS	UV-Visible

PENYINGKIRAN *p*-KLOROFENOL DALAM REAKTOR BIOFILEM KELOMPOK BERTURUTAN

ABSTRAK

Objektif kajian ini adalah untuk membuat perbandingan prestasi di antara reaktor biofilem kelompok berturutan (SBBR) dengan reaktor kelompok berturutan (SBR) dalam biopenguraian p-klorofenol (PCP) dan nitrogen. Reaktor SBR dan SBBR beroperasi dalam tempoh PENGISIAN, TINDAK BALAS (aerobik dan anosik), PEMENDAPAN, PENGELUARAN dan REHAT dalam nisbah masa 2:12:1:1:8 bagi 24 jam satu kitaran. Reaktor SBR digunakan sebagai reaktor kawalan (RC) manakala dua reactor SBBR beroperasi dengan kiub poliuretana sebagai bahan pembawa masing-masing berkepekatan 3 dan 5% (v/v). Usia enapan dikawal selama 40 hari sepanjang eksperimen ini. Prestasi bagi semua reaktor dinilai sebelum dan selepas penambahan PCP dengan memantau kualiti efluen dan ciri-ciri pemendapan enapan. Profil bagi kepekatan PCP, COD, spesies nitrogen, ion klorida, DO dan juga pH ditentukan semasa tempoh TINDAK BALAS. Keputusan menunjukkan bahawa mineralisasi PCP dicapai dengan lengkap dalam semua reaktor dengan kadar penyingkiran PCP dalam SBBR lebih cepat berbanding dengan kadar dalam SBR. Didapati penyingkiran nitrogen ammonia (AN) adalah lengkap bagi ketiga-tiga reaktor sebelum penambahan PCP. Selepas penambahan 100 mg/L PCP, reactor RB1 dan RB2 masih mampu mencapai penyingkiran AN hampir 100% manakala kecekapan reaktor RC menurun kepada 81%. Apabila kepekatan PCP ditingkatkan kepada 300 mg/L, purata kecekapan penyingkiran AN bagi RC, RB1 dan RB2 masing-masing menurun kepada 37, 67 dan 57%. Pada kepekatan 400 mg/L PCP, peratusan

penyingkiran AN menurun lagi kepada 36, 37 dan 40% bagi RC, RB1 dan RB2, masing-masing. Ini mungkin disebabkan kesan ketoksikan PCP terhadap mikroorganisma. Berdasarkan keputusan yang diperoleh, prestasi reaktor dapat disenaraikan seperti berikut: RB2 > RB1 > RC.

p-CHLOROPHENOL REMOVAL IN SEQUENCING BATCH BIOFILM REACTOR

ABSTRACT

The objective of this study is to compare the performance of sequencing batch biofilm reactor (SBBR) and sequencing batch reactor (SBR) in treating *p*-chlorophenol (PCP) and nitrogen. The SBR and SBBR systems were operated in five sequential periods, namely FILL, REACT (aerobic and anoxic), SETTLE, DRAW and IDLE in the time ratio of 2:12:1:1:8 for a cycle time of 24 h. The SBR was used as the control reactor (RC) while the other two SBBRs, RB1 and RB2, were operated with 3 and 5% (v/v) of polyurethane foam cubes as the carrier materials, respectively. Sludge age was maintained at 40 days throughout the study. The performance of the reactors was evaluated before and after the addition of PCP by monitoring the effluent quality and the settling characteristics of the sludge. Profile studies for PCP, COD, nitrogen species, chloride, DO concentrations and pH during the REACT period were also conducted. The results show that complete PCP mineralization was attained in all the reactors with the rate of PCP removal in the SBBRs being faster than that in the SBR. Complete ammonia nitrogen (AN) removal was achieved in all the reactors before the addition of PCP. After the addition of 100 mg/L PCP, reactors RB1 and RB2 still managed to achieve an almost 100% AN removal but the AN removal efficiency for reactor RC deteriorated to 81%. When the PCP concentration was increased up to 300 mg/L, the average AN removal efficiency for reactors RC, RB1 and RB2 decreased to 37, 67 and 57%, respectively. Further addition of 400 mg/L PCP had resulted in AN removal efficiency to deteriorate to 36, 37 and 40% for RC, RB1

and RB2, respectively due to the toxicity effects on microorganisms. Based on the results, the performance of the reactor can be ranked in the following order: RB2 > RB1 > RC.

CHAPTER 1
INTRODUCTION

1.0 Pollution by Organic Compounds

Toxic organic compounds are widely used in processes involving petrochemical, pulp and paper, synthesis of pesticides, tannery and coal refining industries. The development of human industrials and agricultural activities leads to the synthesis of new organic compounds known as xenobiotics (Lora et al., 2000). Toxic organic compounds pose a serious ecological problem as environmental pollutants due to their high toxicity, strong odour emission, suspected carcinogen and mutagen to the living. These compounds were included in the list of priority pollutants because of their toxicity. Microorganisms present in the natural environment are not able to easily catalyse their biodegradation, thus the result is the progressive accumulation of toxic organic compounds in river sediment, groundwater, tissues of organisms and also accumulated in food chain (Droste et al., 1998).

When toxic organic compounds are introduced into the environment, they will give rise to environmental problem. The reported level of toxic organic compounds in the environment ranged from 150 µg/L (Valo et al., 1990) to 200 mg/L (Ettala et al., 1992). According to Grady Jr. (1990) environmental pollution deriving from handling and disposal of toxic organic compounds became a serious problem since the 80s and is considered a threat for the future quality of life. The main reason for the recent situation is the uncontrolled synthesis of xenobiotic compounds and discharged into the environment by industrial activities. Therefore, a highly efficient treatment of wastewater contaminated with these compounds is

required prior to discharge into the environment. Over the years, many studies on the removal of toxic organic compounds in wastewater treatment have been conducted (Kargi and Konya, 2006; Goh et al., 2009; Moussavi et al., 2009).

1.1 Wastewater Treatment

The removal of toxic organic compounds can be achieved by physical, chemical or biological treatment or by a combination of these three treatment processes. Physical treatment such as adsorption and ion exchange only concentrates the pollutants and requires further mineralization by chemical and biological oxidations (Bilgili, 2006; Tang et al., 2007). On the other hand, chemical treatment processes are fast but expensive and may result in the formation of undesirable by-products (Kwon et al., 1999; Yuan et al., 2006; Y. H. Wang et al., 2006b) whereas biological treatment results in complete mineralization and is relatively inexpensive compared to physical and chemical treatment processes.

1.1.1 Chemical Treatment

Among the chemical treatment methods used for phenolic compound removal, chemical oxidation using ozone or Fenton reagent is widely used nowadays. There are limited studies on chemical treatment because many researchers prefer to use a combination of chemical and physical methods due to their ability to effectively destroy the pollutants, rather than chemically oxidizing or physically transferring pollution from one phase to another. Some of the reported chemical treatment of organic compounds are described below.

The treatment of *p*-chlorophenol (PCP) using chemical oxidation at different pH by Fenton's reagent was studied by Kwon et al. (1999). The results showed that pH significantly influenced the degradation of PCP. Almost 100% removal of PCP was achieved at pH 2-4. At pH above 4, the degradation rate significantly decreased due to the decrease of dissolved fraction of iron species. At pH below 2, PCP was not degraded by Fenton's reagent. Hydrogen peroxide at this pH was not decomposed by Fe^{2+} as proven by the constant dissolved oxygen level.

Yuan et al. (2006) investigated the degradation of *p*-nitrophenol (PNP) by cathode reduction and electro-Fenton methods. This study showed that the degradation of PNP was much faster in the cathode cell than in the anodic cell. In the cathode cell, the degradation of PNP was significantly enhanced by the introduction of aeration and Fe^{2+}. The results also showed that more than 98% removal of PNP and about 13% of total organic carbon were removed.

A novel nickel-antimony doped with tin oxide electrode for the electrochemical degradation of PCP was adopted by Wang et al. (2006b). The results showed that the optimal Ni content was at Ni:Sn ratio of 1:500 in atomic ratio in the precursor coating solution, whereas the Sb:Sn ratio was set at 8:500. The charge-based efficiencies were up to 89 μg C^{-1} for PCP destruction and 15 μg C^{-1} for total organic carbon (TOC).

1.1.2 Physical Treatment

Among the several physical treatment techniques in toxic organic compounds removal, adsorption has been widely used for wastewater treatment. A variety of

adsorbents were used such as activated natural zeolites and polymeric resins (Abburi, 2003), amberlite XAD-4 (a non-ionic hydrophobic polyaromatic resin) (Bilgili, 2006), activated carbon fiber (Tang et al., 2007), bentonite and cross-linked polyvinylpyrrolidone. Some of the agricultural solid wastes for instance rockrose, apricot stone, almond shell and cotton stalk have been successfully converted into activated carbon on a laboratory scale. Activated carbon is the most commonly used as adsorbent in wastewater treatment due to its large surface area and affinity for many organic compounds. The disadvantages of physical treatment are that the pollutants are only concentrated and further mineralization by chemical and biological oxidations are required.

Bilgili (2006) used Amberlite XAD-4 resin, a non-ionic macroreticular resin, as the absorbent for PCP at the temperatures of 298, 308 and 318 K. The resin was washed with deionized water to remove inorganic impurities like Na_2CO_3 and NaCl followed by acetone to change its extremely hydrophobic surface area. The equilibrium between PCP and the XAD-4 resin was achieved in approximately 120 min with 90% removal of PCP. The increase in the temperature from 298 to 318 K decreases the adsorption capacity from 22.8 to 20.5 mg/g.

The adsorption of *p*-nitrophenol (PNP) onto activated carbon fibre in simulated wastewater in a batch system was investigated by Tang et al. (2007). It was found that the amount of PNP adsorbed depended on pH, sodium chloride content, adsorbent dosage and temperature. Within 3 min, the uptake of PNP reached 84.8% of equilibrium amount at the adsorbent dose of 4 g/L.

1.1.3 Biological Treatment

Among the physical, chemical and biological treatment processes, biological treatment process was widely used for toxic organic compounds removal due to their low cost and the ability to attain complete mineralization (Kargi et al., 2005; Kargi and Konya, 2006; Carucci et al., 2010). Over the years, the combination of aerobic and anaerobic biological systems for wastewater treatment such as upflow anaerobic sludge blanket (UASB)-activated sludge, aerobic-anaerobic SBR and UASB to trickling filter systems has attracted research interest. The advantages of using biological treatment process are (Schultz, 2005):

a) Low capital and operating costs compared to those of physical and chemical treatment.
b) Oxidation of a wide variety of organic compounds.
c) Operational flexibility to handle a wide range of flows and wastewater characteristics.
d) Reduction of aquatic toxicity.

Disadvantages of biological treatment include excess sludge production, high sludge volume index and inability to treat high concentrations of toxic organic compounds (Tchobanoglous and Burton, 1991; Sirianuntapiboon and Yommee, 2006).

A wide variety of microorganisms are found in wastewaters including viruses, bacteria, fungi, protozoa and nematodes (Horan, 1990). Biological wastewater treatment involves the use of microorganisms (bacteria) to naturally degrade organic waste resulting in BOD and COD reduction, AN removal and wastewater odour control. These microbes produce and release the enzymes needed

to catalyze the chemical reactions that take place when materials decompose. These organisms are competent to adapt and evolve according to whichever contaminant materials are present.

1.2 *p*-Chlorophenol

p-Chlorophenol (PCP) is a very toxic chemical that is introduced into the environment through the discharge of wastewaters originating mainly from chlorophenol production and pulping industries. The specific nomenclature for this substance is 1-hydroxy-4-chlorobenzene and more likely known as *p*-chlorophenol or 4-chlorophenol. The isomers for *p*-chlorophenol are *o*-chlorophenol (2-chlorophenol) and *m*-chlorophenol (3-chlorophenol). The discharge of this compound into the environment is a great concern because of the compound's toxicity and suspected carcinogenicity. It is therefore important to understand some of the basic characteristics of PCP. PCP is in crystal form with a characteristic phenolic odour. It is soluble in water, alcohol, ether and chloroform. The physical and chemical properties of PCP are shown in Table 1.1.

1.2.1 Sources and effects of PCP

Higher chlorophenols (pentachlorophenol and tetrachlorophenol) in large quantities are used in pressure treatment in the wood preservation industry. Lower chlorophenols like monochlorophenol and dichlorophenol serve as intermediates in the production of pesticides, dyes and herbicides (Goel et al., 2010). They are discharged into the environment through various human activities. Wastewater's

Table 1.1: Physical and chemical properties of PCP

Parameter	**Description**
Molecular mass	128.56 g/mol
Form	Crystals
Colour	Colourless
Odour	phenol-like
Melting point	41-44 °C
Boiling point	216-218 °C
Density (45 °C)	1.26 g/cm^3
Solubility in water (20 °C)	27 g/L
Vapour pressure (20 °C)	13 Pa

from these have been reported to cause inhibition on the bioactivity of microorganisms (Monsalvo et al., 2009).

PCP was also reported to accumulate as persistent intermediates during reductive dechlorination of more highly chlorinated phenols (Mohn and Kennedy, 1992). It is used as a denaturant for alcohols as an anticeptic and as a solvent for refining minerals. Besides, PCP is an intermediate by-product in the production of 2,4-dichlorophenol and 4-chlorophenol-o-cresol (Woods et al., 1989). PCP is directly used as wood preservatives, antiseptics and disinfectants or produced as intermediates in the synthesis of dyes, pesticides, biocides and herbicides (Wen et al., 2006). Moreover, PCP can be found in industrial effluents from pulp and paper manufacture, oil refining activities and textile industries (Christiansen et al., 1995; Vallecillo et al., 1999).

The concentrations of PCP found in oceanic waters are around 5-10 ng/L. River waters is noted to have the highest PCP concentrations in the range of 2-2000 µg/L (Micha owicz and Duda, 2007). PCP is also present in drinking water due to the substitution of organic matter and low molecular weight compounds with chlorine atoms.

Human beings are exposed to PCP via ingestion, inhalation or dermal absorption. The common population is thought to be exposed to PCP through ingestion of food and drinking water (Micha owicz and Duda, 2007). Long term exposure of people to PCP in some cases leads to cancer.

1.2.2 Removal of PCP

Treatment processes that are usually used for the removal of PCP in wastewater can be classified under physical (Bilgili, 2006; Hameed et al., 2008), chemical (Y. H. Wang et al., 2006b; Sripriya et al., 2007) and biological processes (Kargi et al., 2005; Kargi and Konya, 2006; Carucci et al., 2010). In addition, PCP removal using a combination of physical, chemical and biological processes has also been reported (Lukác et al., 2007). Biological treatment systems are most widely used in PCP removal and these include, among others, upflow anaerobic fixed-bed reactor (Bali and Sengül, 2003), activated sludge unit (Kargi and Konya, 2006), sequential anaerobic-aerobic reactors (Majumder and Gupta, 2007), rotating brush biofilm reactor (Eker and Kargi, 2010) and aerobic granular sludge sequencing batch reactor and membrane bioreactor (Carucci et al., 2010). Recently, the use of biofilm and membrane reactors has generated great interest among researchers.

1.2.3 Biodegradation of PCP

Biodegradation of chlorinated phenols has been studied by many researchers using pure and mixed bacterial cultures (Hardman, 1991; Hollender et al., 1997; Farrell and Quilty, 1999; Galíndez-Mayer et al., 2008). The degradation of chlorinated aromatic compounds involves two major steps, namely the cleavage of the aromatic ring and the removal of the chlorine atom (Häggblom, 1990). *p*-chlorocatechol (PCC) was identified as the major intermediate during the aerobic degradation of p-chlorophenol (Farrell and Quilty, 1999; Monsalvo et al., 2009) (See Fig. 1.1). Further degradation of PCC led to an accumulation of 5-chloro-2-hydroxymuconic semialdehyde. 5-chloro-2-hydroxymuconic semialdehyde was further metabolized with a stoichiometric release of chloride, indicating complete degradation of PCP.

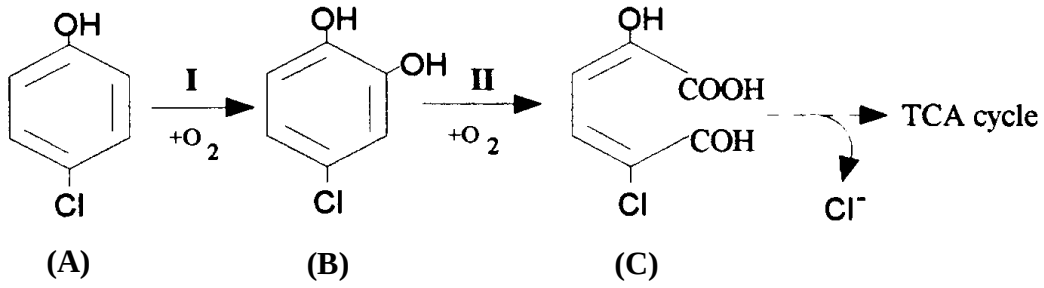

Fig. 1.1: Biodegradation of PCP (Farrell and Quilty, 1999)

(A) p-chlorophenol

(B) p-chlorocatechol

(C) 5-chloro-2-hydroxymuconic semialdehyde

1.3 The Activated Sludge Process

Activated sludge is a type of biological solids that play an important role in the biological wastewater treatment process for removing pollutants. The activated sludge process was discovered in 1913 by two engineers, Edward Arden and W.T.Lockett (Gerardi, 2002). It is a flexible, reliable process that is capable of producing a high quality effluent. Activated sludge has developed into many types of wastewater treatment systems such as conventional, tapered aeration, complete mix, step aeration, contact stabilization, sequencing batch reactor, extended aeration, and pure oxygen systems. A number of factors, namely temperature, pH, wastewater toxicity, aeration time, amount of oxygen available and amount of organic matter available, affect the performance of an activated sludge treatment system (Gerardi, 2002).

1.3.1 Sequencing Batch Reactor (SBR)

Conventional activated sludge systems work generally well for easily degraded components of wastewater but not for hazardous components which are toxic to bacteria or are slow to degrade. The SBR offers an attractive alternative to conventional biological wastewater treatment systems, mainly because of its cost effectiveness and operational flexibility. To date, SBR is the most common activated sludge modification used for industrial wastewater treatment (Rao et al., 2005; Marañón et al., 2008). SBR is a fill and draw (batch) activated sludge system that can be varied by controlling the time period of each cycle. The operational cycle consists of FILL, REACT, SETTLE, DECANT and IDLE steps. The REACT step is adjusted to provide anaerobic, anoxic and aerobic phases in certain number and sequence

when biological nutrient removal is desired (Uygur and Kargi, 2004). Denitrification occurs under anoxic conditions whereas uptake of organic matter and discharge of phosphorus occur under anaerobic conditions. Oxidation of organic matter, uptake of phosphorus and nitrification take place under aerobic condition. Over the years, many researchers used the SBR system for toxic organic compounds and nutrient removal (Tomei et al., 2004; E Sahinkaya and Dilek, 2007; Papadimitriou et al., 2009).

The removal of PCP using a SBR system was studied by Kargi et al. (2005). It was reported that the percent nutrient removals increased with increasing sludge age and decreasing PCP concentrations. The removal of COD and PCP was also investigated and it was reported that the concentrations of PCP to be treated should be less than 900 mg/L in order to obtain high rates of COD and PCP removal at a sludge age of 20 days and hydraulic retention time (HRT) of 25 h (Kargi and Konya, 2006).

Papadimitriou at al. (2009) investigated the treatment of phenol and cyanide - containing wastewater in Continuous Stirring Tank Reactor (CSTR) and SBR activated sludge reactors. An efficient pollutant removal was observed in both systems. However, the performance of the SBR was better than CSTR. The COD concentration in the effluent for the SBR was 360 mg/L corresponding to a removal efficiency of up to 93% while the COD reduction for the CSTR varied between 63 and 92%, yielding the average effluent COD concentration exceeding 600 mg/L. The

addition of powdered activated carbon in the aeration tanks resulted in the optimisation for both systems' performance.

Biodegradation of *p*-nitrophenol (PNP) was investigated by Tomei et al. (2004) in a lab-scale SBR fed with PNP as the sole carbon source. It was observed that complete biodegradation of PNP was easily achieved in the reactor. High removal efficiency and effluent PNP concentrations lower than 1 mg/L were observed for the whole experimental period in the range of feed concentration of 320-400 mg/L of PNP.

The biodegradation of PCP and *o,p*-dichlorophenol (OPCP) separately in batch reactors and mixed in SBR was studied by Sahinkaya and Dilek (2007). It was reported that both PCP and OPCP in batch reactors started to inhibit their own degradation at 53 and 25 mg/L, respectively. The SBR was fed with a mixture of 220 mg/L of PCP, 110 mg/L of OPCP and 300 mg/L of peptone as biogenic substrate at varying feeding periods (0-8 h) to evaluate the effect of feeding time on the performance of the SBR. It was found that, in addition to self inhibition, PCP degradation was strongly and competitively inhibited by OPCP. When the SBR was fed instantaneously (0 h feeding), PCP degradation started only after OPCP was completely removed from the medium. During longer feedings, increased loading rates led to lower chlorophenol concentrations at the end of feeding.

1.3.2 Sequencing Batch Biofilm Reactor (SBBR)

Sequencing Batch Biofilm Reactor **(**SBBR) is the term used to describe reactors containing both suspended growth and fixed film that operate in "fill and draw" mode (Wilderer, 1992). The biofilm provides a long retention time for bacteria and yields less sludge. Application of the SBR strategy to fixed bed biofilm reactors was suggested by Wilderer (1992) to overcome difficulties with respect to growth and maintenance of activated sludge flocs. SBBR is considered to be the hybrid of fully developed SBR technology and biofilm system technology. Recently, there are two types of SBBR that are always used by researchers, namely moving bed reactor and fixed film reactor. Moving bed reactor is a system added with carrier materials which are maintained in suspension by aeration or by mechanical mixing. On the other hand, fixed film reactors are added with carrier materials that are held in one place and do not move. Compared with suspended growth system, the main advantages of biofilm systems are (Moussavi et al., 2009; Yang et al., 2009) :

(a) Greater biomass concentration in the reactor with corresponding higher specific removal rates.
(b) Tolerance to higher concentrations of toxic compounds.
(c) Increased process stability towards shock loadings.
(d) Better effluent quality.
(e) Greater volumetric loads.

Therefore, SBBR systems usually perform better than the SBR and yield high treatment efficiencies.

SBBR is a highly effective biological treatment system that was developed on the basis of conventional activated sludge process and fluidized bed reactor. In the moving bed SBBR, the biomass is grown on small carrier elements that have a lighter density than water and are kept in movement along with the water stream inside the reactor (Chen et al., 2008). The choice of carrier media is a key factor in ensuring the success of a SBBR. Many kinds of carrier have been used in wastewater treatment such as rotating brush (Eker and Kargi, 2006), fibrous carriers (Zhang et al., 2007), functional polyurethane foams (Lai et al., 2008), polyurethane foam cubes, polyethylene rings (Goh et al., 2009), and polystyrene cylindrical (Moussavi et al., 2009).

Biological removal of phenol from strong wastewaters using SBBR was studied by Maussavi et al. (2009). The material used as the carrier media was cylindrical-shape polystyrene pieces (Bee Cell 2000, SANCO) with a specific surface area of 650 m^2 m^{-3}. It was found that the inhibition concentration of phenol in SBBR was 3000 mg/L. The optimum HRT for this system was 40 h, at which the removal efficiencies of phenol and COD were greater than 99%. The reactor was also resistant to shock loads and performed well under various operational conditions.

Goh et al. (2009) compared the performance between SBBRs and SBR in PNP removal. The carrier materials involved in this study was polyethylene rings and polyurethane foam cubes. It was observed that the ammonia nitrogen (AN) removal efficiency for the SBR and SBBR (with polyethylene rings) was 86% and 96%,

respectively, at the influent PNP concentration of 350 mg/L. However, the SBBR (with polyurethane foam cubes) still managed to removed 100% AN. Overall performance showed that SBBRs was better than SBR in PNP and AN removal.

1.4 Biological Nitrogen Removal

During the last few decades, the importance of nutrient removal has increased as a result of the necessity to avoid eutrophication of water bodies receiving untreated waste water and the effluent of waste water treatment plants. From the literature review, many researchers have adopted biological process using activated sludge systems in nitrogen removal because it is very effective and inexpensive (Münch et al., 1996; Kargi et al., 2005; Goh et al., 2009). Biological nitrogen removal consists of nitrification by autotrophs under aerobic conditions and denitrification by heterotrophs under anaerobic/anoxic conditions. Some environmental problems associated with nitrogen containing effluents are (Barnes and Bliss, 1983):

(a) Toxicity of ammonia to aquatic organisms.
(b) Significant oxygen demand on receiving waters due to the oxidation of ammonia to nitrate.
(c) Stimulation of the growth of aquatic plants and algae due to increased nitrogen load.

Conventionally, biological nitrogen removal is accomplished through two processes, namely assimilation and nitrification-denitrification. However, recent

studies showed that simultaneous nitrification and denitrification (SND) has become an attractive technology for nitrogen removal (Guo et al., 2005; K. A. Third et al., 2005). SND occurs within microbial flocs as a result of DO concentration gradients arising from diffusional limitations. That is, there exists anoxic microzones in the center of sludge flocs or in the inner parts of the biofilm that allow heterotrophic denitrifiers to produce nitrogen gas in the traditional way (Puznava et al., 2001). The efficiency of SND depends on dissolved oxygen, the thickness of the biofilm and the influent concentration. A few researchers have investigated SND, sludge quantity and quality in fixed bed sequencing batch reactor mainly in comparison with conventional SBR under the same conditions (Deshuang et al., 2003; Sirianuntapiboon et al., 2005). The effect of temperature on SND via nitrite in a fibrous carrier fixed bed sequencing batch reactor was assessed. It was found that the highest total nitrogen (TKN) removal rate (91.9%) was at 31 °C with DO ranged between 3-4 mg/L.

1.4.1 Assimilation

Nitrogen is assimilated during the growth of all forms of microbes whether heterotrophic or autotrophic. Assimilation is a process which converts ammoniacal nitrogen in the wastewater into the mass of the microorganisms. Assimilation is responsible for removing up to one third of influent Total Kjeldal Nitrogen (TKN) in biological treatment of municipal wastewaters at conventional (non-nitrifying) loading rates (Barnes and Bliss, 1983). In addition, ammonification is the conversion of organic nitrogen to ammonia. Ammonification accompanies the mineralization of organic material during metabolism and occurs in both aerobic and anaerobic processes.

1.4.2 Nitrification

Nitrification is the biological oxidation of ammonia with nitrate as the final product. The nitrifying bacteria obtained their energy by oxidizing the substrates, namely ammonium and nitrite ions (Gerardi, 2002). The reaction requires the mediation of specific bacteria and involves two sequential steps. In the first step, also called nitritation, ammonium is oxidized to nitrite by the action of *Nitrosomanas* microorganisms. The second step is the oxidation of nitrite to nitrate mediated by *Nitrobacter* microorganisms. Both *Nitrosomanas* and *Nitrobacter* are aerobic microorganisms because they develop biochemical activity only in the presence of oxygen (Cervantes et al., 2006). The two steps can be written as:

(1) The stoichiometric reaction for the oxidation of ammonium ions by *Nitrosomanas*:

$$2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$$

(2) The stoichiometric reaction for the oxidation of nitrite ions by *Nitrobacter*:

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- + \text{energy}$$

The overall nitrification reaction is:

$$2NH_4^+ + 4O_2 \longrightarrow 2NO_3^- + 2H_2O + 4H^+ + \text{energy}$$

The population size of *Nitrosomonas* is larger than that of *Nitrobacter* because *Nitrosomonas* obtains more energy from the oxidation of ammonium ions than *Nitrobacter* from the oxidation of nitrite ions. Besides, *Nitrosomonas* has a shorter generation time and is able to increase quickly in numbers as compared to *Nitrobacter*. The difference in generation time affects nitrification and is responsible for the buildup of nitrite ions during unfavourable operational conditions including cold temperature, low dissolved oxygen level, hydraulic washout and toxicity (Gerardi, 2002).

1.4.3 Denitrification

Aerobic stage should be followed by an anoxic stage to complete the removal of nitrogen through denitrification. The biological process of denitrification involves the conversion of nitrate nitrogen to a gaseous nitrogen species, primarily nitrogen gas. These steps start with the conversion of nitrate ions to nitrite ions followed by nitrite ions to nitric oxide (NO) and finally the conversion of nitrous oxide (N_2O) to nitrogen gas. Nitrite ions, nitric oxide and nitrous oxide are considered to be the intermediates. The summary of this process is shown below:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The optimum pH range for denitrification is 7.0 to 7.5. There is a sharp decrease in the denitrification activity for pH values lower than 6 and larger than 8.5. The alkalinity lost during nitrification can be returned to the activated sludge process through denitrification. Denitrification process can only happen in anoxic condition. In general, it has been observed that dissolved oxygen concentration of more than 0.2

– 0.5 mg/L reduces the rate of denitrification significantly. The optimum temperature is between 28 - 35 °C and denitrification is inhibited at wastewater temperature below 5 °C (Gerardi, 2002). Figure 1.2 shows the processes involved in the removal of nitrogen species.

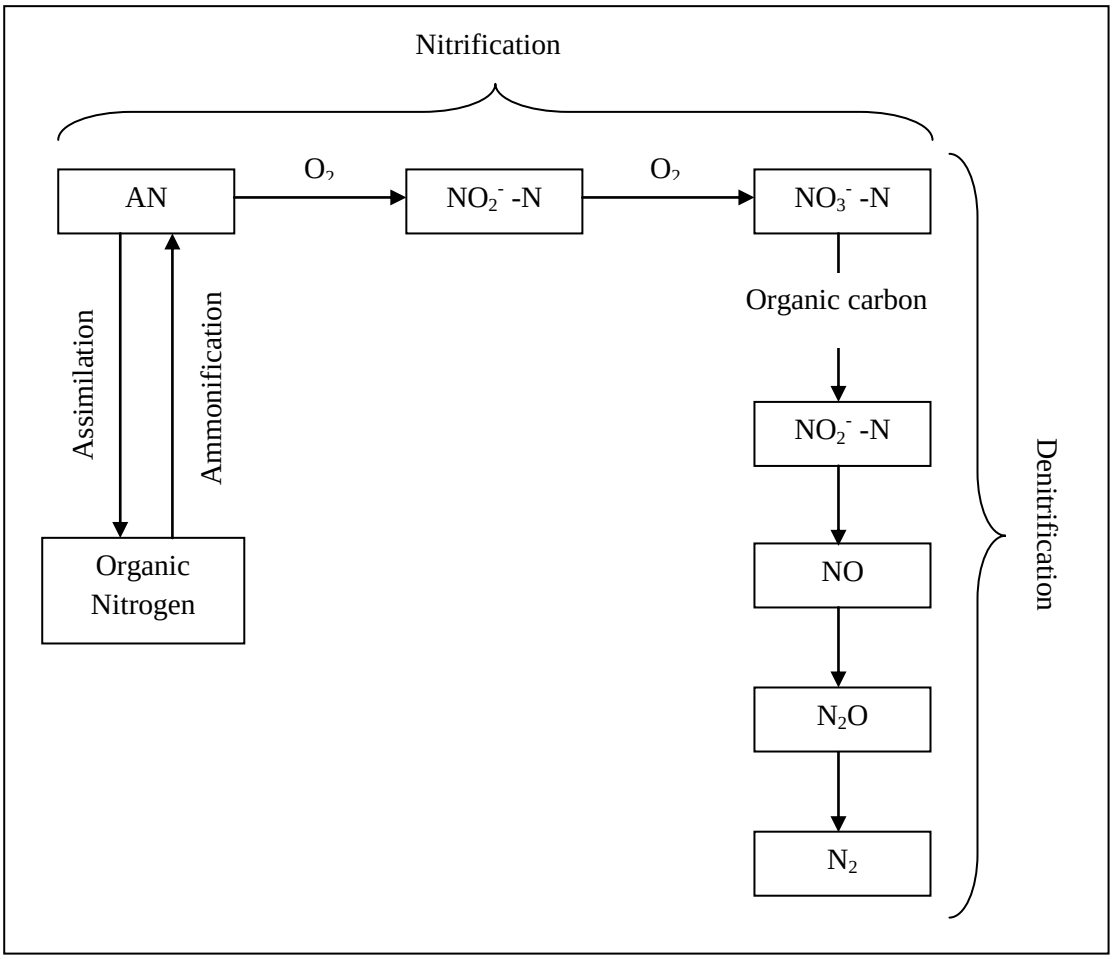

Fig. 1.2: Removal of nitrogen species.

1.5 Objectives

Activated sludge system added with attached growth media has gained its popularity for the removal of toxic organic chemicals in wastewater treatment. The biofilm reactors have been proven to give better performance than activated sludge system and yield high treatment efficiencies (Eker and Kargi, 2006; Moussavi et al., 2009). However, there are few reports on the simultaneous removal of PCP and AN in the SBBR system. In light of the above observation, the objectives of this study are:

1) To compare the performance of SBR and SBBR systems in PCP removal.
2) To study the effect of PCP on nitrogen and COD removal under SBR and SBBR operation.
3) To investigate the kinetics of biodegradation for PCP.

PCP was selected among other toxic organic chemicals because it is widely present in some industrial wastewater and easily degraded as compared to other chlorophenols.

(Grady Jr, 1990; Van Loosdrecht and Jetten, 1998; K. Third et al., 2003; E. Sahinkaya and Dilek, 2005; X. Wang et al., 2006a; Chiu et al., 2007; Rahimi et al., 2010; Chu and Wang, 2011)

CHAPTER 2
MATERIALS AND METHODS

2.0 Experimental

2.1 Sequencing Batch Reactor (SBR) and Sequencing Batch Biofilm Reactor (SBBR)

2.1.1 Experimental Set-up

Three plexiglass reactors, namely a control reactor without carrier materials (RC), a reactor consisting of 3% (v/v) of polyurethane foam cubes (RB1) and a reactor consisting of 5% (v/v) of polyurethane foam cubes (RB2) with the dimensions of 30 x 25 x 20 cm (L x W x H) and a working volume of 10 L were constructed. Three peristaltic pumps (Cole-Parmer) were used for filling in the influent, drawing out the effluent and the addition of organic carbon source (ethanol). In addition, two ejectors were used for agitation and two air stones for aeration. A schematic diagram of the experimental set-up is shown in Fig. 2.1. The sludge from a local municipal sewage treatment plant in Penang was used as the seed culture. The sludge was cultured in the laboratory and acclimatized with the synthetic wastewater (base mix). The difference between SBR and SBBR was that SBBR contained carrier media whereas SBR did not have carrier media.

2.2 Feed Materials

2.2.1 Base Mix

The composition of base mix used throughout this study is shown in Table 2.1. The experiments were conducted using a synthetic wastewater to avoid any fluctuation in the feed concentration, to provide a continuous source of biodgradable organic pollutants and to simulate domestic wastewater (variable from low strength to very high strength) (Rahimi et al., 2010). The stock base mix was

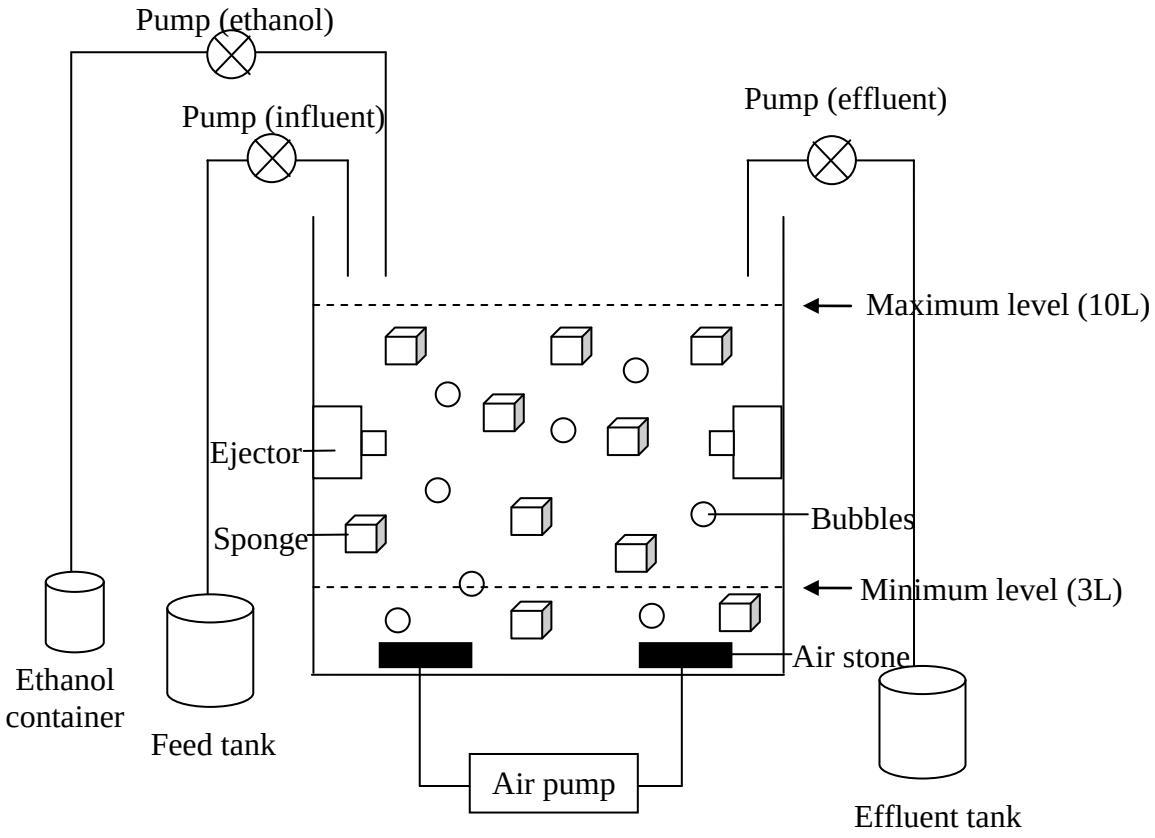

Fig. 2.1: Schematic diagram of the SBBR

Table 2.1: Composition of base mix (Goh et al., 2009)

Compound	Concentration, mg/L
Bacto-peptone	28.1
Sucrose	121.9
KH_2PO_4	35.2
K_2HPO_4	180
$(NH_4)_2SO_4$	226.4
$NaHCO_3$	576
$MgSO_4$	49
$FeCl_3.H_2O$	9.68
$CaCl_2$	41.5

low strength to very high strength) (Rahimi et al., 2010). The stock base mix was prepared once a week and was kept daily in a refrigerator at 4 °C to avoid any decomposition. The working base mix was prepared daily by diluting the stock base mix with tap water and was fed to the reactors by a peristaltic pump. The composition of the feed consisted of 48 mg/L for AN and approximately 160 mg/L COD. The pH of the feed wastewater was nearly 7.60.

2.2.2 *p*-Chlorophenol (PCP) Solution

The desired PCP concentration in the base mix was prepared weekly. A stock PCP solution of 10 g/L was prepared by dissolving PCP in distilled water and stored in an amber glass bottle at room temperature. The PCP was obtained from Merck Schuchardt OHG (Germany) with more than 98% purity.

2.2.3 Carbon Source (Ethanol)

The stock of ethanol solution was prepared by diluting 25 mL of industrial grade ethanol solution with distilled water to a total volume of 500 mL. It was stored in refrigerator at 4 °C using a polyethylene bottle. The ethanol was utilized as an organic carbon source at the beginning of anoxic period.

2.3 Carrier Media

The carrier materials added into reactors RB1 and RB2 served as for the microorganisms. These were polyurethane foam cubes with the dimensions of 1.2 x 1.2 x 1.2 cm (L x W x H). RB1 and RB2 were operated with 3% and 5% (v/v) of polyurethane foam cubes, respectively. A picture of the carrier media is shown in Fig. 2.2. The carrier concentration was calculated based on the percentage from the ratio of the carrier media's volume to the working volume of the reactor. The detailed calculation is presented in Appendix 1.

2.3.1 Carrier Material Characterization

2.3.1.1 Surface Area

The total surface area of a polyurethane foam cube, S_c was calculated based on Equation (2.1), the derivation of which is shown in Appendix 1.

$$S_c = \frac{6V_{pore}}{d} \qquad (2.1)$$

where,

V_{pore} = pore volume, L

d = diameter, m